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# A SURVEY OF LIQUID CHROMATOGRAPHIC-MASS SPECTROMETRIC ANALYSIS OF MERCAPTURIC ACID BIOMARKERS IN OCCUPATIONAL AND ENVIRONMENTAL EXPOSURE MONITORING

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## Abstract

High-performance liquid chromatography/mass spectrometry (HPLC/MS) is sensitive and specific for targeted quantitative analysis and is readily utilized for small molecules from biological matricies. This brief review describes recent selected HPLC/MS methods for the determination of urinary mercapturic acids (mercapturates) which are useful as biomarkers in characterizing human exposure to electrophilic industrial chemicals in occupational and environmental studies. Electrophilic compounds owing to their reactivity are used in chemical and industrial processes. They are present in industrial emissions, are combustion products of fossil fuels, and are components in tobacco smoke. Their presence in both the industrial and general environment are of concern for human and environmental health. Urinary mercapturates which are the products of metabolic detoxification of reactive chemicals provide a non-invasive tool to investigate human exposure to electrophilic toxicants. Selected recent mercapturate quantification methods are summarized and specific cases are presented. The biological formation of mercapturates is introduced and their use as biomarkers of metabolic processing of electrophilic compounds is discussed. Also, the use of liquid chromatography/tandem mass spectrometry in simultaneous determinations of the mercapturates of multiple parent compounds in a single determination is considered, as well as future trends and limitations in this area of research.

### Keywords

liquid chromatography; mass spectrometry; occupational exposure; industrial chemicals; urinary biomarker; mercapturic acid

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Declaration of Interest

The authors hereby report that we have no conflict of interest with the material reported in this paper. The authors alone are responsible for the content and writing of this paper.

## 1. Introduction

The detection and quantitation of urinary mercapturic acids (mercapturates) has become an important tool in characterizing human exposure to occupational and environmental toxicants. Although environmental exposure may be determined by air or water concentrations, estimations of internal dose of a toxicant in individuals based on these external measures are imprecise due to variations in toxicant adsorption and metabolism [1]. Concentrations found in the general environment do not often correlate to an individual's internal dose and various estimates based on models may not be accurate. Determination of urinary mercapturates, which are the products of toxicant metabolism, provide useful biomarkers of individual toxicant absorption and internal dose [2]. The use of mercapturates as biomarkers of toxic occupational and environmental exposure has been extensively reviewed [1, 2] and a survey of HPLC separation and mass spectrometry techniques to quantitate these biomarkers has been undertaken here.

Figure 1 shows a simplified and condensed illustration of mercapturic acid formation [3]. First, glutathione (GSH), an endogenous tripeptide, reacts to inactivate an electrophilic toxicant or toxicant metabolite either spontaneously or by catalysis with glutathione transferase in the liver and in other organs. Next glutamyl and glycine moieties are enzymatically removed to form a cysteine conjugate. This conjugate is, in turn, N-acetylated to form what is generally a toxicant-specific mercapturate [3]. The initial reaction between endogenous GSH and an electrophilic moiety on a compound is regarded as a detoxification step, and prevents reaction of the electrophilic metabolite with cellular components such as proteins, lipids or DNA. The final N-acetylation reaction increases the polarity and hydrophilicity of the metabolite, making it more water soluble and allowing for urinary excretion and elimination [1, 2].

In the interest of brevity, the mercapturate names shown in Table 1 and used throughout this review are truncated from the systematic name of the S-conjugated electrophilic group except in the cases of acrylamide and the triazine and chloroacetamide herbicide derived mercapturates. Thus the systematic name for the mercapturate of benzene, N-acetyl-S-(phenyl)-L-cysteine is shortened to phenyl mercapturate. The abbreviations used for mercapturates vary throughout the current literature, and are generally derived from the S-conjugated electrophilic group. The abbreviations used in this review, as they appear in the cited methods, are listed in Table 1 by parent compound.

This review describes selected HPLC-MS methods for determination of mercapturates as biomarkers in characterizing human exposure to industrial chemicals for the support of occupational and environmental studies. The mercapturate biomarkers considered here are the detoxification products of a wide range of heterogenous electrophilic compounds (Table 1) and no single analytical approach for development of a method for a new specific mercapturate can be recommended. Alternatively, this work is a survey of the multiple analytical approaches described in recently published HPLC/MS mercapturate determinations.

Although Perbellini et al. [3] reviewed methods in 2002 for determining mercapturates in biological exposure monitoring, the scope of their review included gas chromatographic (GC), and high-performance liquid chromatographic (HPLC) methods, including mass spectrometric detection, and it was limited to single mercapurates of benzene, toluene and xylene, as well as the detection of two of the mercapturate products of 1,3-butadiene and of trimethylbenzene [3]. The current review focuses on single and tandem mass spectrometric detection with various modes of HPLC separation on a broader range of mercapturate compounds. Selected representative mercapturate methods, the targeted mercapturate(s), with the labeled internal standards employed and their m/z values or mass transitions for quantitation are summarized in tabular format and highlights briefly described in section 2. An overview of mercapturates as biomarkers of parent compound metabolic activation, as specific indicators of toxicant exposure, and as tools to investigate toxicant metabolism and elimination will be presented in section 3 of the current review. Section 4 will consider simultaneous determination of target mercapturates of multiple parent compounds in a single chromatographic analysis.

## 2. Tabular summaries of selected methods

Tables 2 and 3 summarize some of the numerous HPLC-MS methods reported for the detection and quantification of various mercapturates used in occupational and environmental studies. The terminology and abbreviations appearing in these tables indicate sample preparation techniques, chromatographic conditions, and mass spectrometry detection modes reported for these methods and are explained in more detail in the following sections of this review.

### 2.1. Sample preparation techniques

The specific and reproducible quantification of mercapturates by HPLC/MS presents a series of challenges. Analytes must be separated from substances in the urinary matrix that may interfere with specific detection either by altering the intensity of the analyte signal or co-eluting with the target mercapturate, making sample preparation as critical to success as any other part of the analysis. In the methods reviewed, a variety of sample preparation techniques are described with the simplest being dilution and filtration through 0.2 μ cellulose medium followed by direct injection [12]. Other techniques use sample concentration with re-suspension in methanol or acetonitrile, or protein precipitation by acidification and centrifugation prior to analysis [9, 16, 19, 21]. Most methods utilize manual solid phase extraction (SPE), while four methods utilize online sample extraction with column switching, where urine is passed through a trap using an online multiple valve [5–8]. Small target analytes are retained in the trap. When valve positions are switched, urinary proteins and salts are washed away to waste. A final switching of valves with a change to elution buffer carries analytes from the trap to the LC column for separation. Various SPE media may be employed to extract and concentrate target mercapturates from the urinary matrix: reversed phase (RP), reversed phase-strong anion exchange (RP-WAX) [15] or restricted access medium (RAM) phase online trap cartridges [5–8]. Restricted access media are specialized chromatographic phases that combine size-exclusion with other retention mechanisms. Internal surface-reversed phase RAM combines silica gel particles

having inner pores functionalized to retain small molecules (C18, C8 or C4) with an outer hydrophilic surface such as methyl cellulose.

Online sample extraction using SPE traps with column switching has grown in popularity to create automated analyses that decrease overall analysis time by a substantial reduction in sample preparation steps. This trend is likely to continue to grow in the future as better automated systems become commercially available. Online SPE-LC-MS/MS method design and optimization has been described by Kuklenyik et al. [24] presenting three example applications including a determination of two mercapturates of atrazine. Online SPE-LC-MS/MS has been used extensively by Schettgen and collaborators [25–28] in simultaneous determinations of biomarker mercapturates of aromatic compounds and volatile alkylating agents. Here rapid automated sample extraction and μg/L sensitivity are combined in methods developed for investigation of occupational exposure and environmental exposure in general populations to tobacco smoke and urban air pollutants.

A second form of SPE designed to speed sample through-put uses a 96-well plate format described by Mallet et al.[29] Li et al. [30] and Barbieri et al. [31] used strong anion exchange media in this format to increase extraction throughput in analyses of benzene and toluene mercapturates for studies of benzene exposure in smokers and gas station workers. Recently, Kuklenyik et al. [32] described a 96-well plate sample extraction and sample handling mode used in analysis of four atrazine mercapturates for non-occupational exposure studies. The authors describe rapid optimization of sample extraction parameters, selecting between four extraction eluate compositions to increase analyte stability and maximize MS/MS signal intensity. Following extract elution into 2 μL square wells, further extract transfer and handling were eliminated by evaporation of extracts under nitrogen in the sample wells. Evaporated extracts were stored up to four days without sample degradation before reconstitution immediately before analysis. Like SPE-RAM, this format may be adapted to online analysis when high-throughput and the speed of fully automated analysis are necessary.

## 2.2. Liquid chromatography

**2.2.1. Reversed-phase chromatography—**Mercapturate metabolite analysis methods typically employ reversed-phase (RP) columns having C18, C8 or C4 stationary phases. Commonly used mobile phases contain volatile acids or buffers such as formic acid (HCOOH), acetic acid ($CH_3COOH$), ammonium formate ($HCOONH_4$), or ammonium acetate ($CH_3COONH_4$). Organic modifiers such as methanol (MeOH) and acetonitrile (ACN) are commonly used with either isocratic or gradient conditions for analyte elution. Mixed mode separation has also been reported in the literature. Kotapati et al. [15] combined RP and weak anionic exchange to determine THBMA a mercapturate metabolite of 1,3-butadiene.

**2.2.2. Ultra-high performance chromatography—**Other technological improvements have been made in chromatographic analysis and have been applied to mercapturic acid metabolite determination. Ultra-high performance liquid chromatography (UHPLC) has come into use for biomarker monitoring owing to the fact that most HPLC pump

manufacturers are offering pumping systems capable of maintaining the high back pressure levels required for the technique. Standard HPLC pumping systems have traditionally had maximum pressure levels of approximately 400 Bar (Atmospheres) while UHPLC pumps are designed to handle pressures in excess of 1000 Bar. Alwis et al. [21] developed a chromatographic procedure to determine 24 mercapturate metabolites of 15 VOCs in the human urine using UHPLC. Alwis used a capillary 2.1 mm diameter by 150 mm column containing 1.8 μm spherical C18 bonded silica. Although UHPLC utilizes a smaller particle size and smaller column diameter, it provides sharper peaks while decreasing sample loading over conventional HPLC. The number of theoretical plates should be increased by the use of UHPLC. This in turn may provide greater sensitivity by increasing the signal to noise ratio of the detector. The mass spectrometer is a mass sensitive detector, not concentration dependent such as in ultraviolet detection; therefore, UHPLC may in some cases match or exceed the sensitivity of standard chromatographic systems.

**2.2.3. Hydrophilic interaction chromatography—**One of the most important recent trends in mercapturic metabolite analysis is the use of hydrophilic interaction chromatography (HILIC). RP chromatography is common with electrospray ionization MS (ESI-MS) but has the major limitation in the lack of retention of highly hydrophilic, ionic or polar molecules on the stationary phase. Ordinarily normal phase (NP) would be used to satisfy the separation conditions of highly polar analytes, but NP is not easily made compatible with ESI-MS. HILIC utilizes a polar stationary phase with an aqueous/polar organic solvent mobile phase, where water is introduced to play the role of a stronger eluting solvent. HILIC overcomes the mismatch between the NP like chromatography and the ESI-MS. Hemstrom and Irgum [33] have described HILIC mechanism in great detail, and Nguyen and Schug [34] have described the advantages of HILIC when combined with ESI-MS detection. The application of HILIC in quantitative bioanalysis of compounds of pharmaceutical interest has been described by Jian et al. [35].

HILIC columns have been reported in the literature for the determination of more polar mercapturates [7, 12, 16, 20]. In HILIC separations, mercapturates are separated from urinary matrix by a partitioning mechanism between a water-enriched layer associated with a polar stationary phase and solvent containing ammonium formate or ammonium acetate and 5–15% water to maintain the water-enriched layer on the stationary phase. Since retention increases with hydrophilicity and polarity of the analyte, elution is driven by increasing water content in the mobile phase which is composed of high organic content, usually consisting of acetonitrile or alternatively, methanol.

Biomonitoring of worker exposure to electrophilic alkylating agents, such as acrylamide[36], acrylonitrile [37], and 1,3-butadiene [16, 38], that are carcinogenic, is important in industrial medicine [1, 2]. The mercapturates of these compounds are polar, especially those containing hydroxylalkyl groups that are difficult to retain on RP columns without using highly aqueous conditions and non-volatile mobile phase components. Such conditions are not ideal for analyte ionization by electrospray (ES). Typically HILIC mobile phases using a high organic phase (> 80%) are ideal for ESI-MS analysis, and may enhance ES-MS response [34]. HILIC-ESI-MS appears to be a useful technique, and has been used as a complement to RPLC-ESI-MS studies by Dekant and collaborators [6,7] in

complementary studies of acrylamide and glycidamide mercapturates and other polar acrylamide metabolites (Table 2). Kopp et al. [7] used HILIC-ESI-MS to achieve baseline separation between two acrylamide metabolites, AAMA-sulfoxide and GAMA. Failure to resolve these two metabolites could result in over estimation of urinary GAMA levels, and hence an over estimation of the potential risk of AA exposure in humans. Yan [12] developed a direct-injection method using HILIC to eliminate sample extraction used in earlier methods [10,11] to quantitate the acrolein mercapturate 3-HPMA. The method specificity, linearity, precision and accuracy met required FDA criteria [39]. Sterz et al. [16], and Eckert et al. [20] both employed HILIC chromatography to simultaneously determine multiple hydroxyalkyl mercapturates of alkylating agents. These studies are considered below in section 4 describing simultaneous determinations.

### 2.3. Detection Modes by MS and MS/MS

The basic function of a mass spectrometer is to measure the mass-to-charge ratios (m/z) of analyte ions. Mass spectrometers have various designs which have been reviewed elsewhere in the literature [40]. Although mass spectrometers are capable of scanning for use in qualitative identification of compounds, the monitoring of specific ions for quantitation is the focus of this discussion. For the mercapturic acid metabolite analyses surveyed for this review, single quadrupole (SQ) mass analyzers and tandem mass spectrometers using the triple quadrupole (QQQ) design dominate what is reported in the literature (Tables 2–3). The mass analyzer of the spectrometer has the function to separate the formed precursor ions. Analysis of analytes using single quadrupole instruments are performed in Selected Ion Monitoring (SIM) mode in which only a selected m/z value is detected in the analysis. The majority of the methods found in this review employ tandem transmission quadrupole instruments (MS/MS). In the case of multiple mercapturic acid metabolite analysis, all use the QQQ in which precursor ions are selected in the first quadrupole and allowed to pass into a collision chamber for collision-induced dissociation fragmentation into product ions. Transmitted from the collision chamber, fragmentation product ions will pass into the third quadrupole for detection in the Selected Reaction Monitoring (SRM) mode or multiple product ions may be detected in the Multiple Reaction Monitoring (MRM) mode. The use of tandem mass spectrometry allows for the greatest level of sensitivity and specificity for the analysis method. The increased sensitivity level of the MS/MS detection mode is of particular importance in environmental investigations of ubiquitous low-level industrial pollutant exposures encountered in the general environment where levels of urinary mercapturates are in μg/L or ng/L levels.

## 3. Mercapturates as biomarkers of metabolic processing

The metabolism of electrophilic compounds in individuals varies with toxicant dose, absorption and enzyme polymorphisms [1, 2]. The excretion of mercapturates subsequently varies and their quantitation can provide information about individual occupational and environmental exposure, internal dose and metabolism, including metabolic activation of an electrophilic compound into multiple metabolites many of which may react with GSH to later form a mercapturate. The determination of one or more of the mercapturate products of a parent compound may be required for informative biomonitoring [1, 2]. The metabolism of

some common hazardous chemicals will be described briefly; these include acrylamide, acrylonitrile, 1,3 butadiene, benzene and toluene, and 1-bromopropane. These examples demonstrate broadly how mercapturate determination may be used in biomonitoring and in the investigation of toxicant metabolism and processing in exposed individuals. Toxicant processing may include biological activation, detoxification and elimination of mutagenic or carcinogenic toxicants.

### 3.1. Acrylamide

Acrylamide (AA) is an extensively used industrial chemical intermediate with many applications such as a polymerizing agent in grouts or other acrylamide polymers used in waste water treatment, soil stabilization and paper manufacture [41]. Low levels of acrylamide are present in baked, fired, and roasted foods, and mainstream and sidestream tobacco smoke are common sources of human exposure [42]. Both AA and its oxidative metabolite glycidamide (GA) contain electrophilic groups capable of binding to cellular proteins, a property associated with acrylamide neurotoxicity. Furthermore, glycidamide, a reactive epoxide, binds to nucleophilic nucleic acids to form adducts with cellular DNA and this mechanism is regarded as the cause of AA carcinogenicity [36]. Thus, in human biomonitoring and health risk assessments of AA elimination routes, the metabolism and conversion to GA must be considered. LC/MS analysis have been adapted to these purposes, and four examples of this are listed in Table 2, [4–7]. A method for simultaneous determination of urinary AA and the mercapturate, AAMA, was developed for occupational exposure monitoring [4]. As indicated previously, AA exposure is not limited to the industrial environment. To examine the health risk posed by AA in food, methods for simultaneous quantitation of AAMA and of GA isoform mercapturates, GAMA2 and GAMA3, have been developed and reported in the literature during epidemiologic studies for dietary AA conversion to GA of non-occupationally exposed populations [6,7].

### 3.2. Acrylonitrile

Acrylonitrile (AN) a widely-used industrial chemical and component in tobacco smoke, is another example of a much studied chemical exposure hazard. Acrylonitrile is not directly carcinogenic, but like acrylamide, is potentially carcinogenic through an oxidative metabolite, glycidonitrile (GN) [8, 9]. AN may be detoxified by direct GSH conjugation to form 2-cyanoethyl mercapturate (CEMA) or alternatively AN may be activated to GN that is further metabolized to 2-hydroxyethyl mercapturate (HEMA). Taken together CEMA and HEMA quantitation represent conjugative detoxification of AN, while HEMA quantitation provides a measure of metabolic activation of AN to the reactive epoxide, GN. Similarly, the oxidative metabolism of 1,3-butadiene may result in the formation of 3 reactive epoxides, that if not detoxified by GSH conjugation or other mechanism, can react with nucleophilic sites in DNA and serve as direct-acting mutagens [38]. Their detoxified mercapturates are DHBMA, THBMA, MHBMA1 and MHBMA2 (Table 1). All serve as useful indicators of internal nucleophile exposure and metabolic processing in occupational studies [15].

### 3.3. Benzene and toluene

Benzene and toluene are fairly common solvents encountered in the general environment. These solvents are aromatic hydrocarbons and are frequently used in industrial chemicals, as

common components in fuels and are components in cigarette smoke. Benzene is a known human cancer hazard [43, 44], and the hematotoxicity of benzene has been well elucidated during the past few decades [43, 44]. Both chemicals form corresponding mercapturates which can be used as biomarkers of exposure. Benzene metabolism forms phenylmercapturic acid (PMA) and also forms four other, non-mercapturate metabolites that have been investigated as possible biomarkers, but their urinary levels are influenced by the metabolism of gut flora, diet, and medication use, and smoking [44]. Similarly, the metabolism of toluene forms one mercapturate, benzylmercapturic acid (BMA), and two other non-mercapturate metabolites that are influenced by diet and are not specific for toluene exposure [45]. Because of the desired quality that a biomarker of exposure should be specific for chemical exposure, PMA and BMA that are free of dietary and endogenous interferences are preferred biomarkers for benzene and toluene exposure [44, 45]. However, both may be influenced by smoking. Therefore, in biomonitoring, study subjects should be asked to refrain from smoking for 2 h before urine collection [46].

### 3.4. 1-Bromopropane

1-Bromopropane (1-BP) is an industrial solvent used as a subsitute for chlorofluorocarbons or potential carcinogens in metal electronics degreasing, in adhesives, in aerosol solvents and in dry cleaning [47]. Exposure in workers causes central and peripheral neurological disorders and changes in cellular blood components [48]. 1-BP metabolism, which has been investigated most completely in rodents, produces four mercapturates: one direct GSH conjugate, n-propylmercapturate, and three more derived from detoxification by GSH of oxidative metabolites produced by cytochrome P450 monooxygenase. Oxidative metabolites 3-bromopropionic acid [49], 1-bromo-2-propanol and bromoacetone [50] are in turn conjugated with glutathione to form 2-carboxyethylmercapturate, 2-hydroxypropylmercapturate and 2-oxopropylmercapturate, respectively (Figure 2). Research performed in this laboratory investigated the use of these mercapturates and one oxidative metabolite, 3-bromopropionic acid (3-BPA), as potential biomarkers of 1-BP exposure in highly exposed workers [51]. Urinary mercapturates were quantified by HPLC/MS [13] where the direct GSH conjugate, n-propylmercapturate was predominated in the most heavily exposed worker urine specimens. When the same urine samples were analyzed for 3-BPA using GC/MS [52], no 3-BPA was detected. This result suggests that 1-BP is directly conjugated with GSH, and that oxidative metabolism of 1-BP is not a major metabolic pathway in humans. Studies of human metabolism of 1-BP are limited to analysis of n-propylmercapturate in worker urine [53, 54], and provide no explanation for these unexpected results. However recent studies in rodents by Garner et al. [55], demonstrated that oxidative metabolism of 1-BP by P450 CYP2E1 becomes saturated in highly exposed rats, but not in mice. In rats, 1-BP oxidative metabolism is dose-dependent and becomes blocked with increased toxicant dose. As a result, n-propylmercapturate becomes the predominate urinary mercapturate. When an inhibitor of oxidative metabolism was given to rats, all oxidative metabolites including 3-BPA, were eliminated from their urine, leaving only n-propylmercapturate as the predominate urinary metabolite, mirroring the effect of metabolite saturation of oxidative pathways that likely occurs in high 1-BP exposure. These results suggest a difference between human and rat metabolism of 1-BP and that found in mice. In these studies urinary mercapturate identification was used to investigate toxicant

metabolism, and to deduce possible changes in the activity of up-stream metabolic pathways that occur with changes in the conditions of exposure to toxicants in research animals or in exposed workers.

## 4. Simultaneous determinations of target mercapturates in typical fields

In the past, one of the main technological limitations of mass spectrometers used in HPLC analysis has been the rate of data acquisition and the dwell time of monitoring the response at specific masses. Low data acquisition rates have been known for many years to lead to poor chromatographic peak integration and poor reproducibility of peak area determinations [56]. Rapid data acquisition is necessary in order to minimize chromatographic peak distortion, which can be a problem with multiple analyte methods or spectral data collected from increasingly narrow chromatographic peaks such as with UHPLC. With improvements in data aquisition rate for MS systems, mostly from the advent of much more powerful personal computers, HPLC-MS methods have been more capable of determining multiple mercapturate analytes in a single chromatographic analysis.

### 4.1. Urban air pollutants

Simultaneous determination analysis is well suited to investigate complex exposures to volatile organic compounds (VOC) in occupational settings, in exposure of urban populations to air pollutants, and in cigarette smoke [57–59]. Sabatini et al. [22] developed a simultaneous determination for the mercapturates of benzene, toluene and xylene (BTX) using FDA validation guidelines [39] to measure BTX co-exposure in traffic wardens exposures to automobile exhaust and urban air pollutants examining the urine of men and women, including smokers and non-smokers (Table 3). To study urban populations exposed to cigarette smoke, Wu et al. [9] used an ultra-high performance small bore column to resolved structurally similar mercapturates of acrylonitrile, CEMA and HEMA. UHPLC was used ambitiously by Alwis et al. [21] to determine 24 mercapturate metabolites of 15 VOCs in the urine of multi-ethnic males and females including both smokers and non-smokers. Target mercapturates of this study included a 3rd isomer of 1,3-butadienemercapturate, 4-hydroxy-2-buteneylmercapturate (MHBMA3). Although multiple analyte analysis is an obvious modern trend, the rate of data acquisition must be kept in mind when developing and validating new biomarker analytical methods.

### 4.2. Alkylating agents

The mercapturates of many alkylating agents contain highly polar hydroxyalkyl groups and are difficult to retain on RP media. To address this difficulty, Eckert et al. [20] developed a HILIC method to determine 6 hydroxyalkyl mercapturates (HAMA), including the first reported determination of 2,3-dihydroxypropyl mercapturate of glycidol (Table 3). In agreement with earlier reports [28, 58], Eckert reports only 1 MHBMA peak in urine of smokers. Most recently, Sterz et al. [16] combined UHPLC with HILIC to separate 1,3-butadienemercapturate isomers, MHBMA1 and MHBMA2 in human urine (Table 3). Altogether, further studies are needed to better evaluate 1,3-butadiene metabolism. Perhaps, as in the complementary studies of acrylamide by Dekant and collaborators [6, 7], RPLC-ESI-MS/MS and HILIC-ESI-MS/MS may be used to investigate the isomers of MHBMA,

and evaluate their utility as biomarkers in occupational exposure, in smokers, and in urban populations.

### 4.3. Herbicides

Herbicides are widely used resulting in exposures in commercial, agricultural, urban and residential settings. Sensitive, automated high throughput methods for occupational and environmental biomonitoring of atrazine (ATZ) exposure have been developed by Barr and collaborators [23, 32, 60]. These methods allow simultaneous determination of urinary ATZ, ATZ metabolites, their mercapturates and hydroxylated derivatives by combining RP-hexyl phenyl chromatography with APCI ionization and multiple precursor-product ion monitoring (RP-APCI-MRM). Panuwet et al. [60] adapted this analysis to water and urine samples, adding online SPE extraction and concentration of analytes, to determine ATZ, two ATZ mercapturates, and four other ATZ metabolites. Kuklenyek et al. [32] expanded Panuwet's online method to create a two-dimensional HPLC analysis that incorporates SAX and RP chromatographic separation modes using three multiple-port valves and three pumps. This system allows determination of ATZ and 11 ATZ derivatives, including 4 mercapturates for toxicology and occupational exposure applications. Norrgran et al. [23] applied RP-APCI-MRM analysis with a manual mixed-polarity polymeric SPE preparation of a 2-ml urine sample to determine multiple herbicides: phenoxyacetate ATZ; 3 chloroacetanilide herbicides acetochlor, alachlor, metochlor, and their mercapturates. The method achieves LODs < 1 μg/L, sufficiently sensitive to detect exposures in non-occupationally exposed general populations (Table 3).

## 5. Conclusions

HPLC-MS provides a powerful and useful tool for mercapturic acid quantification. HPLC-MS allows for a high level of specificity in the analysis method and for the elimination of interfering or co-eluting substances found in a biological matrix such as urine. Tandem (MS/MS) offers the greatest level of specificity for analysis and will likely be the predominant technique utilized for many years to come. The advances in separation techniques, such as mixed mode or HILIC, will likely become more widely used as well as the utilization of UHPLC as pumping systems become more available commercially and MS systems become more versatile and more powerful. Recent applications of HPLC-MS/MS to the simultaneous determination of multiple mercapturates as indicators of exposure and metabolic processing in individuals may be expected to provide useful information for estimating exposure risk in both occupational and environmental health studies.

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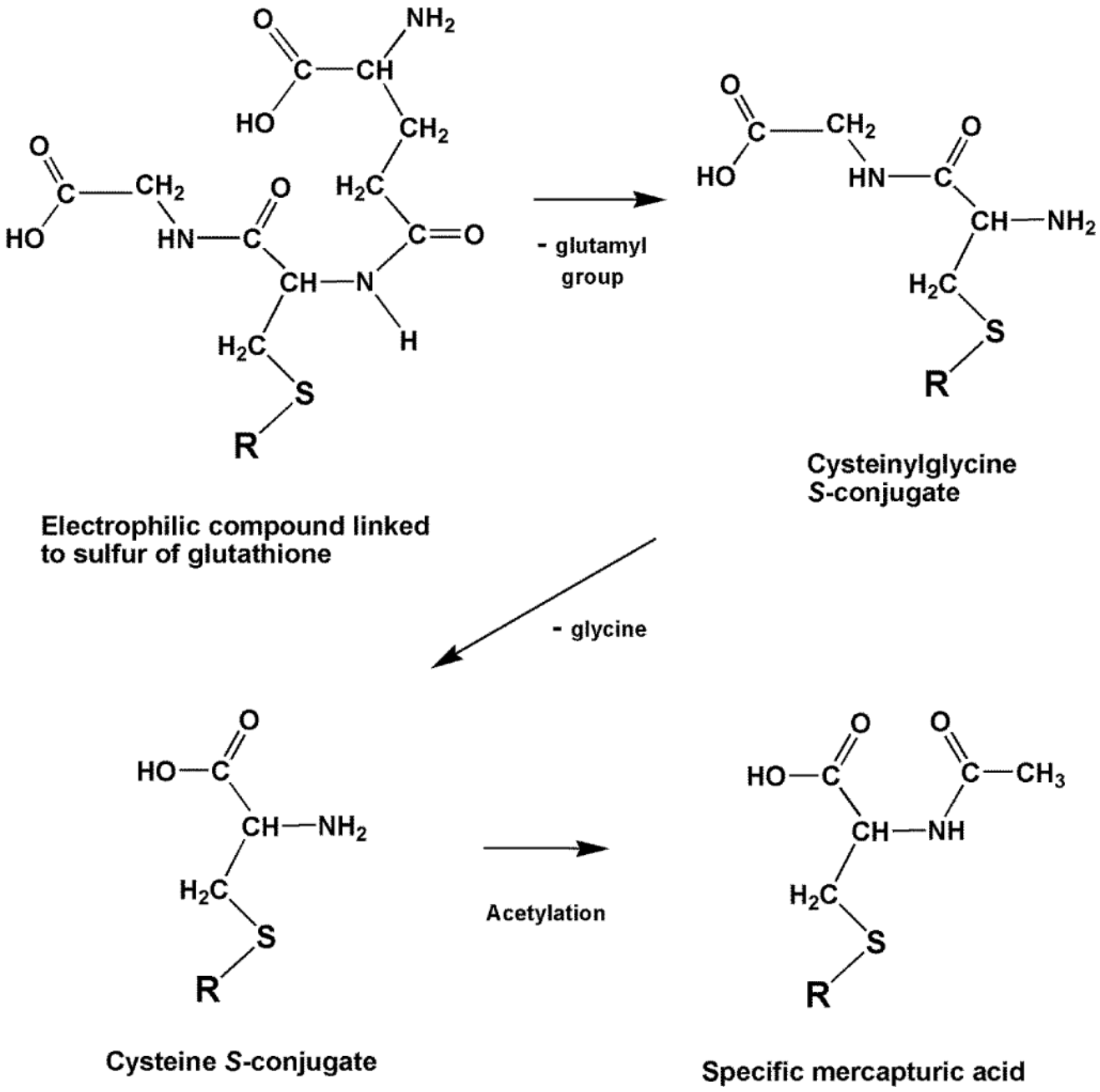


**Figure 1.**

Mercapturate formation begins by conjugation of glutathione with an electrophilic group (R) of a toxicant or toxicant metabolite with the sulfur of glutathione in the liver or other tissue to form a glutathione *S*-conjugate. Glutamyl and glycine moieties are removed to form a cysteine *S*-conjugate that is *N*-acetylated to form a specific mercapturate. Adapted from Perbellini et al. [3].

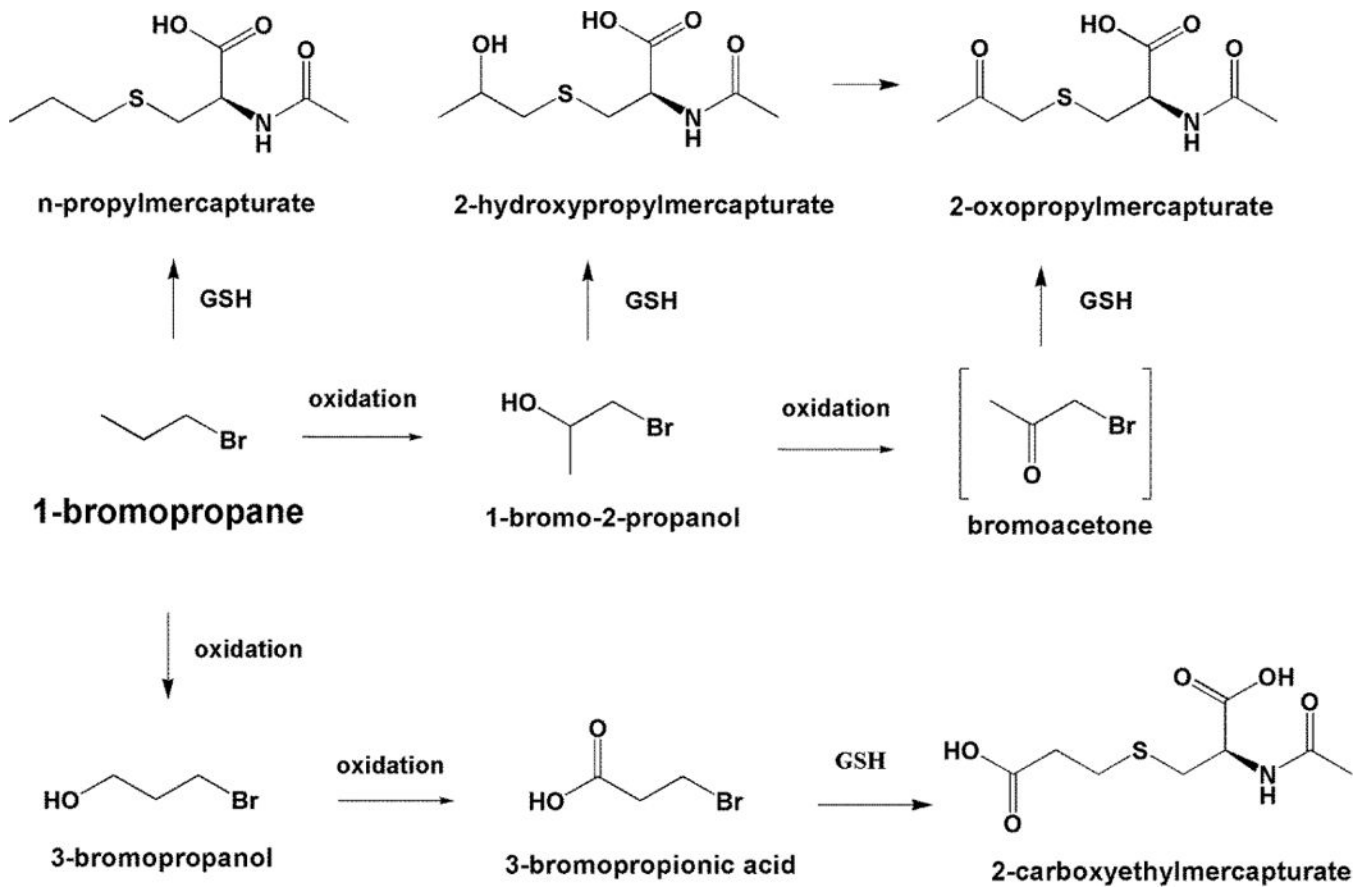


**Figure 2.**

Metabolism of 1-bromopropane in the rat by multiple pathways. 1-bromopropane may be directly conjugated with glutathione (GSH) to form *n*-propyl mercapturate, or 1-bromopropane may be oxidized to form metabolites which are conjugated with GSH to form other mercapturates.

## Table 1

Mercapturate abbreviation and common name by parent compound.

| parent compound | abbreviation | *N*-acetyl-*S*-(R)-cysteine |
|---|---|---|
| acrylamide | AAMA | 2-carbamoylethyl mercapturate |
| | GAMA2 | 1-carbamoyl-2-hydroxyethyl mercapturate |
| | GAMA3 | 2-carbamoyl-2-hydroxyethyl mercapturate |
| | NASPC | *s*-propionamide mercapturate |
| acrylonitrile | CEMA | 2-cyanoethyl mercapturate |
| | HEMA | 2-hydroxyethyl mercapturate |
| acrolein | 3-HPMA | 3-hydrosypropyl mercapturate |
| atrazine | AZMA | atrazine mercapturate |
| acetochlor | ACMA | acetochlor mercapturate |
| alachlor | ALMA | alachlor mercapturate |
| metolachlor | MEMA | metolachlor mercapturate |
| benzene | PMA | phenyl mercapturate |
| 1-bromopropane | NPMA | *n*-propyl mercapturate |
| 1,3 butadiene | DHBMA | 3,4-dihydroxybutyl mercapturate |
| | THBMA | 2,3,4-trihydroxybutyl mercapturate |
| | MHBMA1 | 1-hydroxymethyl-2-propenyl mercapturate |
| | MHBMA2 | 2-hydroxy-3-butenyl mercapturate |
| crotonaldehyde | HPMMA | 3-hydroxypropyl mercapturate |
| dimethylacetamide | AMMA | acetamideomethyl mercapturate |
| dimethylformamide | AMCC | *n*-methylcarbamoyl mercapturate |
| ethylene oxide | HEMA | 2-hydroxyethyl mercapturate |
| glycidol | DHPMA | 2,3-dihydroxypropyl mercapturate |
| styrene | PHEMA | 1-phenyl-2-hydroxyethyl mercapturate |
| tetrachlorethylene | TCVMA | trichlorovinyl mercapturate |
| trichlorethylene | 1,2-DCVMA | 1,2-dichlorovinyl mercapturate |
| | 2,2-DCVMA | 2,2-dichlorovinyl mercapturate |
| toluene | BMA | benzyl mercapturate |
| xylene | DPMA | 2,4-dimethylphenyl mercapturate |
| | MBMA | *o*-methylbenzyl mercapturate |

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**Table 2**

LC/MS analysis of mercapturic acids in human urine.

| Parent compound | Sample preparation | Chromatography | Interface/detection | Target mercapturate | m/z or mass transition | Limit of detection | Reference |
|---|---|---|---|---|---|---|---|
| Acrylamide | SPE | RP, gradient CHOOH/ACN | ESI/SQ/SIM$^-$ | AAMA<br>$d_4$-AAMA | 233<br>237 | 200 μg/L | [4] |
| | RP-pretrap column switching | RP, gradient CHOOH/ACN | ESI/QQQ/MRM$^-$ | NASPC<br>$^{13}C_3$-NASPC | 233/104<br>236/107 | 5 μg/L | [5] |
| | RP-pretrap column switching | RP, isocratic CHOOH/ACN | ESI/QQQ/MRM$^-$ | AAMA<br>$^2H_3$-AAMA<br>GAMA2<br>$^2H_3$-GAMA2<br>GAMA3<br>$^2H_3$-GAMA3 | 232/104<br>236/104<br>249/73<br>252/73<br>249/120<br>252/120 | 0.5 μg/L<br>1.0 μg/L | [6] |
| | RP-pretrap column switching | HILIC, gradient $CH_3COOHNH_4$/ACN | ESI/QQQ/MRM$^-$ | AAMA<br>$^2H_3$-AAMA<br>GAMA2<br>$^2H_3$-GAMA2<br>GAMA3<br>$^2H_3$-GAMA3 | 232/104<br>236/104<br>249/73<br>252/73<br>249/120<br>252/120 | 0.5 μg/L<br>1.0 μg/L | [7] |
| Acrylonitrile | RAM phase column switching<br>protein precipitation | RP, gradient CHOOH/ACN<br>RP, isocratic CHOOH/ACN | ESI/QQQ/MRM$^-$<br>ESI/QQQ/SRM$^-$ | CEMA<br>$^2H_3$-CEMA<br>HEMA<br>$^2H_3$-HEMACEMA<br>$d_3$-CEMA<br>HEMA<br>$d_4$-HEMA | 215/162<br>206/77<br>210/81<br>215/86<br>215/162<br>217/165<br>206/77<br>209/82 | 1 μg/L<br>30 μg/L<br>0.05 μg/L<br>0.05 μg/L | [8]<br>[9] |
| Acrolein | SPE | RP, isocratic CHOOH/ACN | ESI/QQQ/MRM+ | 3-HPMA<br>*N*-acetyl-cys | 222/163<br>162/122 | 50 μg/L | [10] |
| | SPE | RP, gradient $CH_3COOHNH_4$ /MeOH | APCI/QQQ/SRM$^-$ | 3-HPMA<br>$^{13}C_3$-3-HPMA | 220/91<br>237/105 | 0.9 μg/L | [11] |
| | dilute, filter inject | HILIC, gradient $CHOOHNH_4$/ACN | ESI/QQQ/MRM$^-$ | 3-HPMA<br>$d_3$-3-HPMA | 220/91<br>223/91 | 22 μg/L | [12] |
| Bromopropane | SPE | RP, gradient $CH_3COOH$/MeOH | ESI/SQ/SIM$^-$ | NPMA<br>$d_7$-NPMA | 204<br>211 | 10 μg/L | [13] |
| 1,3-Butadiene | SPE | RP, gradient $CH_3COOH$/MeOH | ESI/QQQ/MRM$^-$ | DHBMA<br>$d_7$-DHBMA | 250/121<br>257/128 | 3.6 μg/L | [14] |
| | SPE | RP/WAX, gradient $CH_3COOH$/ACN | ESI/QQQ/SRM$^-$ | THBMA<br>$d_3$-THBMA | 266/137<br>269/137 | 0.1 μg/L | [15] |

| Parent compound | Sample preparation | Chromatography | Interface/detection | Target mercapturate | m/z or mass transition | Limit of detection | Reference |
|---|---|---|---|---|---|---|---|
| | concentrate/resuspend in MeOH | HILIC, gradient $CH_3COOHNH_4$/ACN | ESI/QQQ/MRM$^-$ | 1-MHBMA<br>$d_6$-1-MHBMA<br>2-MHBMA<br>$d_6$-2-MHBMA | 232/73<br>238/77<br>232/103<br>238/109 | 0.05 μg/L<br>0.24 μg/L | [16] |
| Dimethyl-acetamide | SPE | RP, gradient $CH_3COOH$/MeOH | ESI/SQ/IonTrap | AMMA<br>AMCC | 223<br>219 | 1500 μg/L | [17] |
| Dimethyl-formamide | SPE | RP, isocratic $CH_3COOH$/ACN | ESI/SQ/SIM$^-$ | AMCC | 219/162 | 2 μg/L | [18] |
| | protein precipitation | RP, isocratic HCOOH/MeOH | ESI/QQQ/MRM+ | AMCC | 221/122 | 4 μg/L | [19] |

ACN: acetonitrile, ESI: electrospray ionization, HILIC: hydrophilic interaction chromatography, MRM: multiple reaction monitoring, RAM: restricted access medium, RP: reversed phase, SIM: selected ion monitoring, SRM: selected reaction monitoring, SPE: solid phase extraction, SQ: single quadrupole, QQQ: triple quadrupole,

APCI: atmospheric pressure chemical ionization, MeOH: methanol, RP-WAX: mixed mode reversed phase-weak anionic interaction, *N*-acetyl-cys: *N*-acetyl-cysteine

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**Table 3**

LC/MS simultaneous determination of multiple mercapturic acids

| Parent compounds | Sample preparation | Chromatography | Interface/detection | Target mercapturate | m/z or mass transition | Limit of detection | Reference |
|---|---|---|---|---|---|---|---|
| multiple determination | SPE | HILIC, gradient $CH_3COOHNH_4$/ACN | ESI/QQQ/MRM$^-$ | | | | |
| glycidol | | | | DHPMA<br>$^{13}C_2$-DHPMA | 236/107<br>238/107 | 5.5 μg/L | [20] |
| ethylene oxide | | | | HEMA<br>$d^4$-HEMA | 206/77<br>210/81 | 4.0 μg/L | |
| propylene oxide | | | | 2-HPMA | 220/91 | 7.0 μg/L | |
| acrolein | | | | 3-HPMA<br>$d_3$-3-HPMA | 220/91<br>223/91 | 3.0 μg/L | |
| 1,3 butadiene | | | | DHBMA<br>$d_7$-DHBMA<br>MHBMA<br>$d_6$-MHBMA | 250/121<br>257/128<br>232/103<br>238/109 | 4.5 μg/L<br>5.0 μg/L | |
| multiple determination | protein precipitation | RP, gradient $CH_3COOHNH_4$/ACN | ESI/QQQ/MRM$^-$ | | | | |
| crotonaldehyde | | | | HPMMA<br>$^2H_3$-HPMMA | 234/105<br>237/105 | 2.0 μg/L | [21] |
| styrene | | | | PHEMA<br>$^{13}C_6$-PHEMA | 282/153<br>288/159 | 0.7 μg/L | |
| tetrachlorethylene | | | | TCVMA<br>$^{13}C_2$-TCVMA | 290/161<br>294/165 | 3.0 μg/L | |
| trichlorethylene | | | | 1,2 DCVMA<br>$^{13}C$-$^2H_3$ 1,2-DCVMA<br>2,2 DCVMA<br>$^{13}C$-$^2H_3$ 2,2-DCVMA | 257/127<br>261/127<br>257/127<br>261/127 | 12.6 μg/L<br>6.5 μg/L | |
| xylene | | | | DPMA<br>$^2H_3$-DPMA | 266/137<br>269/137 | 5.0 μg/L | |
| multiple determination | SPE | RP, gradient $CH_3COOH$/MeOH | ESI/QQQ/MRM$^-$ | | | | [22] |
| benzene | | | | SPMA<br>$d_5$-SPMA | 238/109<br>243/114 | 0.30 μg/L | |
| toluene | | | | SBMA<br>$d_5$-SBMA | 252/123<br>259/123 | 0.35 μg/L | |
| xylene | | | | MBMA<br>2-HPMA | 266/137<br>220/91 | 0.40 μg/L | |

| Parent compounds | Sample preparation | Chromatography | Interface/detection | Target mercapturate | m/z or mass transition | Limit of detection | Reference |
|---|---|---|---|---|---|---|---|
| multiple determination | SPE | RP, gradient $CH_3COOH$/ACN | APCI/QQQ/$MRM^-$ | | | | [23] |
| atrazine | | | | AZMA<br>$^{13}C_3$-AZMA | 343/214<br>246/217 | 0.060 μg/L | |
| acetochlor | | | | ACMA<br>$^{13}C_6$-ACMA | 351/130<br>357/130 | 0.048 μg/L | |
| alachlor | | | | ALMA<br>$^{13}C_6$-ALMA | 409/280<br>294/165 | 0.039 μg/L | |
| metolachlor | | | | MEMA<br>$^{13}C_6$-MEMA | 365/162<br>371/168 | 0.036 μg/L | |

ACN: acetonitrile, ESI: electrospray ionization, HILIC: hydrophilic interaction chromatography, MRM: multiple reaction monitoring, RP: reversed phase, SPE: solid phase extraction, QQQ: triple quadrupole,

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